# HUMAN INFESTATION STUDIES IN PORTO RICO BY THE EGG COUNTING METHOD.

THESIS

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## Introduction.

The accumulation of evidence in regard to the distribution of hookworm disease and the development of control measures have demanded more and more refined methods for the study of its dissemination. These methods are needed to increase our knowledge of the aetiology of the disease, to determine what control measures are best suited for particular localities and for evaluating the results of control work. The attempt to limit the spread of hookworm disease is being conducted on a world-wide scale and the amount of work to be done is overwhelming in comparison with the available resources, so the needed information must be obtained by the use of methods of the greatest possible simplicity and economy.

Of the information needed for adequate hookworm control work, that which relates to the degree of human infestation is of great importance. Four methods have been used in attempts to obtain this information, viz., (1) physical examination to determine the clinical symptoms produced by the hookworm, (2) haemaglobin determinations to estimate the degree of anemia, (3) the counting of the number of worms expelled after some standardized treatment and (4) the ~~examination~~ estimation of the eggs in the stools of infested individuals. The last two of these methods which are attempts to directly measure the number of parasites present and their potential dissemination, will be considered in this paper.

A significant question in this connection is whether in preliminary surveys it is either necessary or practicable to measure accurately the amount of illness due to hookworms. It would certainly be desirable but it was found a very difficult and complicated task by the Uncinariasis Commission to the Orient (see Darling, Barber and Ashbur, 1920) and few surveys have the facilities which were at their disposal. In the control campaign which follows the preliminary survey the treatment of illness as such is incidental and does not involve the difficulties which beset measures for the prevention of the dissemination of hookworms. These measures form the foundation of the campaign and should receive the major part of the effort. Accurate information in regard to the factors concerned in the spread of the parasites is more readily obtained than is a measure of the illness due to hookworms. An important part of this information depends on a knowledge of the degree of human infestation. The worm counting method of Darling has been utilized by Darling, Saillie and others in determining the degree of infestation by stating it in terms of worm index, viz., the average numbers of worms harbored by a sample of the population.

By the use of this technique important information has been secured on the relative value of different anthelminthics, (Darling, Barber and Ashbur, 1920; Darling and Saillie, 1921; Caius and Khashur, 1920-1922) on the distribution of the different species of

human hookworms (Darling, 1920), on the worm index of various population groups (Darling, 1921; Smillie, 1922 a) and on the evaluation of the results of control measures (Smillie, 1922 b). In addition these studies have added a considerable body of information on the aetiology of hookworm disease under tropical conditions.

Discussion of the Darling Worm Counting Technique.

The following description of the worm counting technique is given by Smillie (1922 b).

"The worm count method was that of Darling .....In brief it consists in choosing a group of 25-30 people, representing all ages, both sexes, various occupations, etc., and giving them a massive dose of a vermifuge which removes 96-99% of the hookworms harbored. Chenopodium, individed doses of 5 cc is usually given, preceded and followed by a heavy saline purge. All stools are saved for a period of 48 hours, and worms are picked out, counted and classified. The worm count method has a disadvantage in that only small groups of people can be examined. To be effective the group must be representative, and great care must be taken in the choice of individuals, so that a true cross section of the community may be obtained. Young children cannot be included because of the vigorous nature of the treatment, but this is not a serious source of error since we have already shown that hookworm infection is seldom a serious factor in the lives of the old or very young."

There can be no question that this method is the most accurate now available for the determination of the infestation of individual patients, especially in those cases in which the drastic treatment is not contraindicated by concomitant pathological conditions. Darling and Smillie have also shown that it can be used more safely and with quite accurate results with smaller doses of the anthelmintic.

The following disadvantages are inherent in the method:

1. The numbers of persons who can be examined at a reasonable cost in any preliminary survey is so small that it is extremely difficult to obtain a sample which will fairly represent the population of any large community.

2. Certain classes of people are automatically eliminated from consideration in the sample because of the impossibility of obtaining cooperation in the careful saving of stools.

3. Even those who consent to cooperate require the most rigid supervision by responsible officers. The technique is best carried out in a jail with each patient locked in a separate cell but this condition is not compatible with true sampling of the population.

4. All errors of this method except those due to sampling are in the same direction. Failure to collect all of the stools for the required time, failure of the anthelmintic to act with full efficiency, contraindication of the full dose of anthelmintic, carelessness of assistants in the preliminary washing of the stools, all tend to give less than the true number of worms.

5. The relative costs of various methods of survey cannot be directly compared but it is evident that this method is very expensive.

In discussing methods for preliminary hookworm surveys of new fields, Smillie (1922 a) says, "In making a hookworm survey of a new field the fact that interests us most is not the per cent of individuals infected with hookworms, but the percent of individuals who have hookworm disease. This information can be determined most accurately by the worm count method; that is, by treating considerable numbers of people and counting their worms. The worm count method is a tedious and difficult process, however, and cannot be adopted as a routine procedure."

To be practicable for general use, a test must be simple, so that a considerable part of the routine work can be performed by subordinates. It must be capable of application to a fair sample of the population with a reasonable expenditure of time and money. It must be conducted in such a manner as to set no precedents that will interfere with a subsequent control campaign. The worm count method meets the first of these conditions but presents weakness in the other two. It is very expensive and time consuming in proportion to the number of people examined. The radical treatment used in the early surveys tends to prejudice the people against the remedy, even if there are no accidents. As frequently carried out, the worm count method involves the payment of patients for their services. This precedent, used among primitive people, produces a serious obstacle to subsequent treatments

The Use of Stoll's Egg Counting Method.

In view of the difficulties involved in the use of the worm counting method an attempt was made in Porto Rico to determine whether the method devised by Stoll (1923 a and 1923 b) for counting parasites' eggs in feces could be used to measure the human infestation factor in population groups. In considering the possibilities of the use of this method in field studies it should be kept in mind that it is just as important to know the numbers of eggs which are being deposited on the soil by hookworm infested individuals as it is to know the numbers of worms present, since this information will form the basis for studies of the dissemination of the parasites and for planning control measures.

In our Porto Rican studies, therefore, the egg counting method of Stoll was used in connection with epidemiologic investigations of hookworm disease in five areas.

Four of these areas were in the valley of which Utuado is the chief town and were chosen to include as many as possible of the variations in environment and living conditions which were to be found in the rural portion of the valley. The other area was about 30 miles away and was chosen to study the effect of a routine campaign of treatment on the factors under investigation. The present paper will deal with the results obtained by the egg counting method in these areas. The rest of the data from these researches will be considered in later numbers of this series.

In each of these areas a census was taken and an effort was made to examine every person. This effort was so successful that the few persons who did not submit specimens may be left out of account. Each person received a two-ounce ointment box with a label on the cover to serve as a container for the fecal specimen. Due precautions were taken to prevent the mixing of the containers in large families of illiterates by issuing two or three at a time and by collection a few hours later. The containers were brought to the central office on the day of collection and on the same or the following day the number of ova per gram of feces was estimated as follows:

Each specimen was given a number corresponding with the case number of the patient. Two large test tubes were given the same number. Into each test tube there was placed three grams of feces weighed from the container by difference. One-tenth normal sodium hydroxide was added to make a volume of 45 cc. and a few glass beads were dropped in. Then the tube was closed with a rubber stopper and vigorously shaken until the material was well comminuted. The weighing and shaking were usually done by well trained technical assistants, but as counting was entrusted to subordinates. After shaking, the tube was not allowed to remain stationary for more than a few seconds before the portion to be examined was removed. A sample, 0.15 cc., of the thoroughly mixed suspension was placed on a microscopic slide and covered with a large cover glass (22 x 40 mm.). All of the suspension under the cover glass was carefully examined under a 16 mm. objective and all of the hookworm eggs were counted. The results gave the number of eggs in 0.01 grams of the specimen. The

duplicate suspensions, which were made from each specimen, were counted by different observers and the average accepted as the true value. The accuracy of these counts has already been discussed by Stoll (1923 a and 1923 b).

Description of the communities studied by the egg counting method.

The areas which were included in this study were designated as A, B, C, D, and E. In addition to the counts on these five areas, the series of egg counts presented in this paper include counts made in area C after a treatment campaign and 40 counts from miscellaneous cases from around Utuado. Before presenting the analysis of these data it will be necessary to briefly describe the areas.

Area A. This area was a part of a small village located near the coast. The inhabitants were laborers on a nearby sugar estate, small farmers and beggars. With one exception all the houses included in this area were provided with latrines. The people who lived at this house claimed that they used the latrine at the house next door. These latrines were quite generally used. There were, however, several important foci of soil pollution and soil infestation and some scattered pollution. These latrines had all been installed in connection with the sanitary campaign conducted one year before our study. Prior to that time none of these houses had latrine provision. This area had been very slightly influenced by treatment. Of the 40 people on whom egg counts were made none had

been treated within five years and only a few reported that they had been treated six to ten years before. The census of the nine homes considered was 41, the number on whom egg counts were made was 40. Of these, 36 were positive with an average egg output of 4,060 per gram.

Area B. This area was located near Utuado in close proximity to the former "Anemia Hospital." It had been under the influence of the treatment activities which had been carried on in Utuado from 1904 to the present. About 20% of the people reported that they had received a recent course of hookworm treatment, i.e., within three years, and the majority reported that they had been treated. Although no systematic sanitary campaign had been carried on, more than half the houses had latrines. At the other houses soil pollution was prevalent. The census of this area gave 119 names. Of these 111 were examined, 93 being found positive. The egg count gave an average of 1,050 hookworm eggs per gram.

Area C. This area which was about ten miles from Utuado consisted of a closely packed group of houses on a sugar and coffee estate. The people were laborers on the estate and on nearby farms. Nearly all of the men and many of the women worked in the coffee during the picking season. There had been little influence of any treatment campaign although severe cases of anemia were sometimes taken to a dispensary several miles away for treatment. Not a single individual in our census had been treated within three years, and only

a small number reported any previous treatment. There was a great deal of severe clinical hookworm disease, and much anemia as shown by haemoglobin estimates. There was no pretense of sanitation, only one house having a latrine in use, and soil pollution and soil infestation of the grossest kind were prevalent in banana clumps, and portions of coffee and cane fields adjacent to the area. The census from this area showed 95 persons, of whom 92 were examined with 88 positives. The hookworm egg count gave an average egg output of 7,740 per gram.

Area C after treatment. In this area a campaign of treatment was instituted and egg counts were made two weeks after the last treatment. The treatment was with oil of chenopodium on the basis of 1.5 cc. for a strong adult and corresponding doses for children. Three treatments, one week apart were given to all patients available for them. The number examined after treatment was 89. Of these 42 were still positive with an average egg count of 630 hookworm eggs per gram.

Area D. This area consisted of the houses on a typical coffee estate. The people on this estate had been influenced by campaigns of sanitation and treatment, but not such a large proportion had received treatment as in area B. About 10% of the people of this area had received hookworm treatment within a three year period and a total of about 25% reported that they had at some time received treatment. Of the twenty-one houses included in this area ten had

latrines. At all the others and at a few of these provided with latrines there was soil pollution near the houses, and at some of them both soil pollution and soil infestation were very gross. The census from this area was 100. Of these 96 were examined, with 86 positives and an average egg output per gram of 1040 hookworm eggs.

Area B. This area consisted of a number of houses on a coffee estate at some distance from Area D but in the same general type of environment, as Area D. This area had been influenced less than Area D by hookworm control work both in treatment and sanitation. About 8% of the people examined in this area reported that they had been treated within three years and about 25% reported previous treatments some, however, dating back as far as 30 years. Sanitation in Area B was much poorer than in Area D or Area E, since only five out of the seventeen houses had latrines and at only one house were there no signs of soil pollution. The census from this area was 93. Of these 85 were examined with 76 positives and an average egg output of 2930 per gram.

Miscellaneous cases. There are also included in these studies the egg count data from certain cases who reported at the central office for medical advice. These cases were all inhabitants of the general region studied, but were not from any of the special areas. A few were clinical cases of hookworm disease

but the majority showed no characteristics which would differentiate them from the general population of the region. The number of such cases examined was 40 of which all were positive with an average egg output of 3800 eggs per gram.

Analysis of data according to age and sex.

It has seemed worth while first to analyse the total data obtained from the egg counts in Porto Rico, except, of course, the counts made after treatment in Area C, with relation to the egg output in the various age groups and according to sex. In comparing the analysis so obtained with similar analyses made elsewhere it should be remembered that infestations in any particular place are modified by local conditions and particular habits of the people. We must therefore be extremely cautious in drawing generalizations from any particular set of data. One peculiarity of our Porto Rican areas is that except in Area C, a considerable influence had been exerted both by treatments and sanitary work, so that the data represent a modification from the original condition. For this reason in certain connections Area C, which was practically uninfluenced by control work, will be considered separately and compared with the other areas.

Table I and figure I show the data arranged according to the output of eggs in ten year age groups and according to sex. It will be seen from the curves that during young adult life there is a slightly greater output in males than in females, and that this difference is quite significant between twenty-five and forty-five, although in later years the females surpass the males. It

Table I.

Egg output of Porto Rican Cases by age and sex.

| Age group | Males | | Females | | Total | |
|---|---|---|---|---|---|---|
| | No of persons examined | Mean egg output per gram of feces | No. of persons examined | Mean egg output per gram of feces | No. of persons examined | Mean egg output per gram of feces |
| 0 - 9 | 71 | 1200 | 65 | 820 | 136 | 1020 |
| 10 -19 | 55 | 3640 | 51 | 4100 | 106 | 3870 |
| 20 -29 | 45 | 3870 | 37 | 3000 | 82 | 3460 |
| 30 -39 | 25 | 4780 | 32 | 2780 | 57 | 3660 |
| 40 -49 | 19 | 3220 | 20 | 4540 | 39 | 3900 |
| 50 + | 18 | 6180 | 18 | 9990 | 36 | 8090 |
| Total | 231 | 3220 | 225 | 3290 | 456 | 3250 |

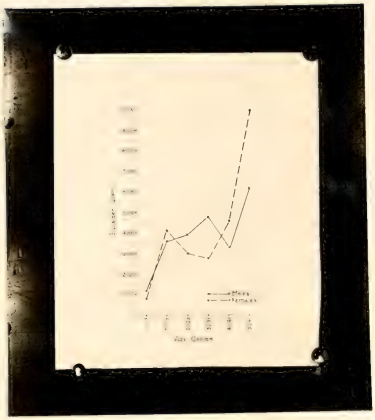

Figure I. Egg output of the Porto Rican cases with relation to age and sex.

is of interest in this connection to separate our data and plot the curve for Area C separately. It will be seen that in Area C the egg output of the females is greater than that of the males (Fig. 2) while in the other areas (Fig. 3) the males have the highest averages in almost all age groups. The curves for the age groups are characterised by a rapid rise to about 19 years, a plateau extending to about 45 years and a sharp rise in the older groups.

A comparison of our data with the Brazilian cases of Emilio (1922 a) in regard to age and sex distribution gives some interesting information. The two sets of data give much the same picture of the acquisition of the hookworm infestation in the early years of life. If in the Porto Rican series the average egg output of persons fifteen years or more in age who had had no treatment within three years, be taken as the expected output of adults, the increase in output of children with increasing age may be studied. There were in all the areas 267 persons in age groups of 15 years or more. Thirty-two of these had had treatment within 3 years. The remaining 235 untreated cases had an average egg output of 4240 eggs per gram of feces. The age groups of children with the average egg output of each group and the ratio of this output to the average adult output is shown in Table II. This shows a gradual rise in the egg output during the second to the eighth years and a very marked increase occurring between the eighth and eleventh years with the average output very nearly to that of the average adult by the completion of the fifteenth year.

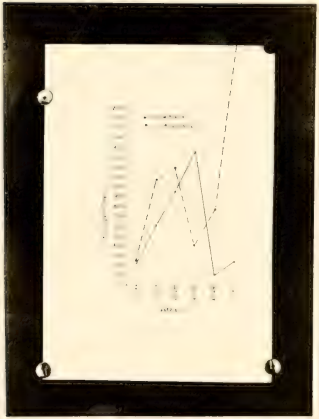

Figure II. Egg output of the Area C cases with relation to age and sex.

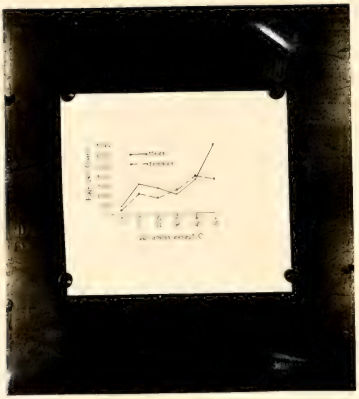

Figure III. Egg output of all areas except Area C with relation to age and sex.

Table II. Comparison of egg output of children of Porto Rican areas with average egg output of adults. Similar comparison of hookworms harbored by children and adults of the Brazilian cases given by Smillie.

| Age Group | Porto Rico Cases | | | Brazil Cases | | |
|---|---|---|---|---|---|---|
| | Number of Individuals | Average Egg Output | Percent. of Adult Output | Number of Individuals | Average Number of hookworms | Percent. of Average no. harbored by adults. |
| 0- 3 | 21 | 1.2 | 2.5 | | | |
| 4- 5 | 31 | 6.8 | 14.0 | 6 | 0.5 | 0.3 |
| 6- 7 | 40 | 11.5 | 24.0 | 16 | 18.4 | 10.3 |
| 8- 9 | 20 | 12.9 | 26.7 | 32 | 42.8 | 28.8 |
| 10-11 | 23 | 35.5 | 773.3 | 36 | 117.4 | 78.9 |
| 12-13 | 22 | 27.0 | 56.8 | 49 | 149. | 100.0 |
| 14-19 | 14 | 45.7 | 94.4 | 79 | 159.4 | 107.1 |
| Total | 180 | | | 221 | | |

Similar data have been obtained in regard to the increase in the number of hookworms harbored by Brazilian children from Smillie's studies (Smillie 1922, a). The average number of worms harbored by 514 cases of 16 years or older was 142.8. The average number of hookworms harbored by members of the different age groups and their ratio to the average number harbored by adults is given in Table XI. Figure IV shows graphically the data from Table XI. The acquisition of hookworms is apparently begun later in the areas studied in Brazil but once begun the parasites are acquired more rapidly. On the whole, however, the two curves are comparable and harmonize quite as well as could be expected of data obtained from different countries, in groups with different age distributions and by different techniques.

A further comparison of the data from the Brazilian cases with the Porto Rican cases shows certain significant differences in age and sex distribution.

If figure I be compared with figure V, which represents a summary of worm counts on 562 cases in Brazil (Smillie, 1922 a), it will be seen at once that there must have been considerable differences in the forces operating to maintain the infection through life. In each there is the initial rise in early life which has already been noted. In the Brazilian cases the worm index in males rose much higher than that of the females. While each tended to show a plateau through middle life, the values represented by these plateaus were far apart. In the Porto Rican cases the difference

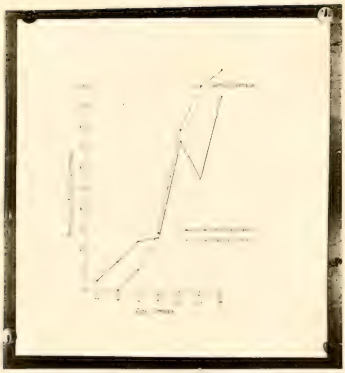

Figure IV. Comparison of the Porto Rican cases with Smillie's Brazilian cases with regard to the degree of hookworm infestation in the early years of life.

Figure 1. Smillie's Brazilian cases (Smillie, 1922a, p. 7, Chart 1) showing hookworm infestation in relation to age and sex.

between the egg output of males and females in middle life was not nearly so great and was not constant. In Area C the output of the females of these groups was larger than that of the males. In the other areas the output of females was less than that of the males.

In the cases studied in Brazil there was evidence that infection ceased or was greatly reduced at the end of the active period of life of the agricultural laborer or at about 45 years, for from this time the number of worms decreased. This was not true of the egg output of the older age groups in Porto Rico. There was a marked increase in egg output in later life.

The explanation of the difference between the Brazilian and the Porto Rican results is probably to be found in differences in the habits of the people. Smillie's interpretation of his results indicates that the chief source of infection in the Brazilian areas which he studied is excrement deposited in the fields. All the evidence collected in Porto Rico tends to show that infested spots near the homes are at least as important as are spots in the field. Regularly visited spots of soil pollution were found at distances varying from twenty yards to one hundred yards from the houses. The places chosen were obscured from public view by banana plants, coffee, cane, or other vegetation. They were often on very steep hillsides and they were visited only for purposes of defecation and for work in connection with the cultivation of the field in which they were located. The

evidence showed that these spots were visited by adults and the older children. Children younger than six or eight years apparently had no definite places for defecation and were not accustomed to go more than a few yards from the houses for that purposes. It is probable that this habit accounts for the gradual rise in egg output of the younger children of these areas shown in Figure IV and that the steeper rise in the years eight to eleven is due quite as much to visiting polluted spots near the houses as it is to working in the fields. Field work by children under twelve was not a conspicuous feature in Porto Rican industrial conditions. Compulsory education keeps these children in school during nine months of each year.

The difference between males and females in the Porto Rican series cannot be explained solely on the basis of the field work of the males, for the field work is generally shared by both sexes during the coffee picking season when ground itch is most prevalent. It must be explained in part by the greater modesty of a considerable part of the female population in the young adult age groups who avoid frequent visiting of the spots of pollution by the use of bed-room utensils. They are also less likely than the men to retain on their feet the mud which adheres during visit to the infested spots. The greater infestation of females than of males in Area 3 is probably due to the great poverty of the community, lack of utensils, heavy infestation of population and of the soil. The women of this area probably visited the infested spots more frequently than the men and were observed to be careless in their

habits of cleanliness. In this case it appears that the men were actually saved from a certain amount of infestation by their absence in the fields during the greater part of the day.

The Brazilian cases showed a reduction in worm index in the later years of life and it is stated that they may be omitted from consideration without serious error (Avilla, 1922, b, p. 30). This generalization does not hold for the cases examined in Porto Rico for the egg output showed a distinct rise in the older people. The apparent reason for this increase is that these people no longer work in the field and are brought in closer and more frequent daily contact with the concentrated foci of soil infestation around the houses. Ashford's and Gutierrez's (1911) description of the gradual increase in infestation of the persons sick with hookworm disease may well be applied to these old people, "We believe that we should emphasize the fact that it is usually in the immediate vicinity of his home, and even sometimes when the floor is of earth, inside the hut that the jibaro is infected. He defecates 'en el montecito', or as we should say, 'in the bushes,' and this montecito has become a synonym for privy. Owing to weakness due to the disease itself or to indolence, his excursions to the montecito become shorter and shorter and result in a general pollution, by himself and his family, of a considerable area around his home."

A Comparison of the egg output in the different areas examined.

The individual areas may be compared by separating each into arbitrary groups according to egg output. The limits of outp t

of the groups selected are as follows:

1. Persons found negative. A more critical technique might have shown a small output by some of these cases.

2. Output of 1-599 eggs per gram of feces.

3. " " 600-2099 eggs per gram of feces.

4. " " 2100-5099 " " " " "

5. " " 5100-11099 " " " " "

6. More than 11,100 " " " " "

Study of the distribution of the patients into these groups shown in Table III and figure VI gives an idea of the relative infestation of the areas. It is obvious from the inspection of the table and the bar diagrams that very little information is obtained by comparing these areas on the basis of the percentages of positives and negatives. While these percentages agree in a general way with the other data presented the variation is not sufficient to make a striking comparison. The percentage of positives varies from 79.3 in area B to 95.7 in area C. If, however, we arrange these positives in arbitrary groups according to egg output we note at once that there is a wide variation in the grouping. In area B, with only 20.7 % of negative cases there are nevertheless 77% which showed an output of less than 600 eggs per gram of feces. In area C only 21.7% of the persons examined were in this group. Areas A and D had practically the same percentage of negatives, 10 and 10.4 respectively, but in area A only 30% had an output of less than 600 eggs per gram of feces while in Area D 47.0% had an output within these limits.

Table XXI

Comparison of Egg output of Porto Rican Cases by Areas.

| Egg output per gram of feces | Area A | | Area B | | Area C | | Area D | | Area E | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Persons | Per Cent | No. of Persons | Per Cent | No. of Persons | Per Cent | No. of Persons | Per Cent | No. of Persons | Per Cent |
| 0 | 4 | 10.0 | 25 | 20.7 | 4 | 4.3 | 10 | 10.4 | 8 | 7.2 |
| 1 - 599 | 4 | 10.0 | 51 | 45.9 | 16 | 17.4 | 36 | 37.5 | 35 | 39.8 |
| 600 - 2099 | 12 | 30.0 | 22 | 19.8 | 15 | 16.3 | 23 | 24.0 | 22 | 25.8 |
| 2100 - 5099 | 10 | 25.0 | 9 | 8.1 | 23 | 25.0 | 17 | 17.7 | 9 | 10.8 |
| 5100 -11099 | 5 | 12.5 | 5 | 4.5 | 18 | 19.6 | 9 | 9.4 | 9 | 10.8 |
| More than 11,100 | 5 | 12.5 | 1 | 0.9 | 16 | 17.4 | 1 | 1.0 | 4 | 4.8 |
| Totals | 40 | | 111 | | 92 | | 96 | | 85 | |

Figure VI. A comparison of the egg output in the different areas studied.

It is probably more useful to select an arbitrary egg output which can be regarded as being undoubtedly significant in dissemination of the parasites if the feces are deposited on the soil, or if we choose to think in terms of mass infestations indicates a significant number of worms in the individual case, and compare the areas by the percentage of persons falling in this group. If we choose 2100 eggs per gram as an arbitrary number which must be significant, the percentages in the group with an output in excess of this amount are shown in the dimension lines on figure VI. It will be shown later that the minimum egg output in this group probably indicates a worm index of between 80 and 140. The percentages in this group in increasing order are: Area B, 13.6; Area E, 26.6; Area D, 28.1; Area A, 50; Area C, 62.0.

These figures are in harmony with the known conditions existing in the areas which were discussed in the description of the areas. Area B had long been influenced by a nearby dispensary. Areas D and E were similarly influenced but much less strongly. Area A had had no treatment but natural conditions were not so favorable to the spread of hookworms as in the mountains. Area C had had no treatment of importance, no sanitation, and nature had provided excellent conditions for the survival of the hookworm larvae. In comparing these areas it will be seen that the factor which seems to be of the greatest significance in influencing the degree of infestation was the amount of previous treatment.

The immediate effect of treatment on egg output as shown in Area C.

After the egg counts on area C were completed a campaign of treatment was instituted to determine the effect of the mass treatment technique on the egg output. Sixtyeight cases were examined two weeks or more after the last treatment and the average egg output was 630 per gram of feces. The average egg output before treatment was 7740 so the percentage reduction in egg output was 91.9.

This is somewhat lower than the reduction in the worm index of 95-98% anticipated by Darling and Saillie from the use of their technique but the difference was probably due to the following factors:

1. The area was extraordinarily heavily infested and it is well known that heavy infestations are not removed so readily as light ones.

2. There were a number of heavily infested individuals who were clinically ill and were unable to undergo treatment by the standard technique. This applies also to the heavy infestations which occurred in children and old people.

3. Not all of the patients took three treatments, some because of reluctance, some because it was necessary to discontinue treatment on medical grounds. Re-examinations were made regardless of whether one, two or three treatments had been taken. These considerations make it seem certain that the egg counts after treatment in area C were really an accurate measure of the reduction in worm index. The reduction in specific age groups is shown in Table IV

and Figure VII and in the bar diagrams of Fig. VI the distribution of the patients according to egg output is compared with the distribution before treatment.

Evaluation of information obtained by egg counts.

The data given above show that the estimation of the egg output has demonstrated differences in the various groups which are in harmony with conclusions reached from study of their history, habits, and sanitation. The study of Area C before and after a treatment campaign showed differences in egg output which are within the anticipated range. Therefore it appears that the application of this method to a community gives us averages which are valid measures of the potential dissemination of the parasites. This is the essential information required for the guidance of control measures.

If the worm index is desired, it is highly probable that further experience with this method will give it with sufficient accuracy for routine work. It has been shown by Stoll (1923b) that in cases studied by him in Porto Rico, the egg output bore a definite relationship to the number of female worms harbored. The ratio varied with the concentration of the fecal material, from about 44 eggs per gram per female worm in formed stools to 12 eggs per gram per female worm in diarrhoeic stools. These ratios were shown to be very close to estimates made by earlier writers on the subject. Smillie (1922, b, p. 65) has shown in a careful study of 279 cases in Brazil that the numbers of male and female worms are approximately equal. Therefore if the ratio between eggs per gram and female worms harbored can be determined the total number of worms will be known

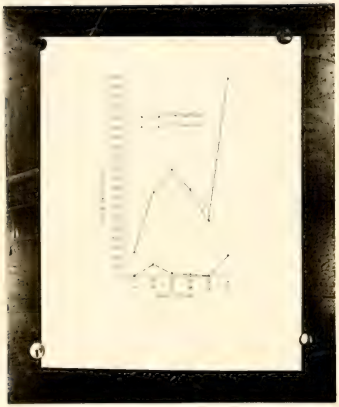

Figure VII. Egg output of Area C before and after treatment according to age groups.

within reasonable limits. Further studies are needed to determine whether these ratios hold in other countries and under conditions other than those which existed in Porto Rico. The application of these ratios to the data from our areas gives worm indices which are in agreement with previous worm counts made in Porto Rico.

The number of worms in Stoll's cases can be estimated as between 0.04 and 0.07 times the number of eggs per gram for formed and for very soft feces respectively. The factor for diarrhoeic feces need not be considered. If these factors be applied to the data from our cases we obtain measures of the probable high and low limits of the worm index of the groups.

Reduction of egg output to probable worm index by areas.

| Group | Eggs per Gram | Low Limit of Worm Index | High Limit of Worm Index |
|---|---|---|---|
| Area A | 4060 | 162 | 284 |
| Area B | 1050 | 42 | 74 |
| Area C | 7740 | 310 | 542 |
| Area D | 1840 | 73 | 129 |
| Area E | 2530 | 101 | 177 |
| Area C after treatment | 630 | 25 | 44 |
| Heaviest case | 120500 | 4820 | ----* |

*This specimen was desiccated so the factor for formed feces is the one which applies.

Reduction of egg output to probable worm index in groups in Table VI.

| Eggs per Gram | Low Limit of Worm Index | High Limit of Worm Index |
|---|---|---|
| 600 | 24 | 36 |
| 2100 | 84 | 147 |
| 5100 | 204 | 357 |
| 11100 | 444 | 777 |

Since in Porto Rico any group of stools examined contained both formed and soft types, the correct factor to be used in obtaining the worm index from our egg count would lie somewhere between the two extremes of 0.04 and 0.07.

Worm counts made on seventy cases in another mountain district of Porto Rico in 1906 by Ashford, Gutierrez and Lieg (1911) showed from 73 to 8403 hookworms with an average of 1029. These, however, were clinical cases confined in the hospital for treatment. In the series of ten cases in which worm counts were made by Stoll the numbers found were from 37 to 1154, average 470. Worm indices determined from egg output can only be accepted with caution until further studies are made to determine the variation in the conversion factors under different conditions of climate, race, diet, and distribution of the two chief species of hookworm, N. americanus, and A. duodenale.

Even if it should be found that the variations are too great to allow conversion of egg output into worm index, still the egg output will remain the measure of the concentration of the infectious material or of the potential spread of the disease.

Men who have had experience in hookworm field work have noted many cases in which the difficulty of finding hookworm eggs by the usual technique was out of proportion to the clinical severity of the case. From this ground they argue that the output of eggs is so variable that it cannot be used as a measure of infestation. It has been shown of some of the previous methods of estimating the number of ova that they failed when used for comparing individual cases. The purpose of a survey is not the comparison of individuals but the comparison of communities and the results of the present study indicate that in such a survey the individual variations disappear in the averages. An error which is sometimes made is the assumption that a certain number of parasites should mean a certain train of symptoms. This is far from being the case as has been shown by many writers, notably by Darling, Barber and Hacker in their work in the East, where they found that "Individual cases of infection could not be properly compared on account of the unknown factor of individual resistance or perfection of defense. But when all the cases were talked and averaged it was observed that there was some correlation between degrees of anaemia and numbers of worms."

Discussion of Practical Details of the Application of the Egg Count Method in Surveys.

The possibility of using the egg count method in preliminary surveys seems to us to be a very important application. The extent of a survey is usually limited by duration and cost. The use of

the egg count method in a survey should require very little expenditure in excess of that required for the usual survey by positives and negatives except the salaries of additional technical assistants. Two assistants are frequently employed in the usual survey and it is suggested that five be employed to carry out the egg count method. This allows for a supervising technician, three microscopists, and an assistant. It is estimated that such a staff could make accurate counts on about fifty specimens a day, duplicate counts being made of each specimen. It would be necessary for the medical officer to give careful training and thorough supervision to his technical staff. Previous experience has demonstrated that the application of certain simple administrative procedures in handling a carefully chosen native technical staff will usually get reliable results. If we allow one month for training, a three months' survey would enable the examination of at least two thousand specimens. If a trained staff could be moved from one survey to another the number of specimens which could be handled in each survey would be greatly increased. It will be seen that the speed here estimated is not so great as that given by the positive and negative method, in which two thousand specimens are often examined in a two months' survey with only two microscopists. It is our opinion that the additional information gained by the egg count method will be of sufficient use in the application of control measures to justify the increased cost.

It must be understood that it is not intended that the egg counting shall supplant any part of the work of a survey except the

microscopical technique which is now used. It might even be desirable to retain the older technique for use on specimens found negative by the egg counting method, since the latter has not yet been demonstrated to be so critical as the centrifugation or the flotation technique in the detection of light infestations.

It is significant to note here that Dr. R. B. Hill has recently made a series of human infestation studies in Porto Rico in which he trained his regular microscopical staff to make the egg counts. His results are in essential agreement with those just given and illustrate the feasibility of utilizing native microscopists in making surveys by this method. Dr. Hill's results will be published in the next number of this series.

We wish here to make acknowledgement to Dr. R. B. Hill for his help in collecting specimens and data from Area A and to Mr. N. R. Stoll for his aid in adapting the egg counting method to the conditions of the study. Thanks are also due to Mr. B. L. Augustine and Dr. Florence K. Payne for help and suggestions during the course of this investigation.

Summary

1. A quantitative estimate of the human infestation factor and the potential dissemination of the parasite is important in understanding the aetiology of hookworm disease.

2. The larling worm count method, while giving an accurate determination of the degree of human infestation in individual cases is difficult to apply as a routine procedure.

3. The egg count method devised by Stoll to determine the number of parasite eggs present in a given quantity of feces was used in Porto Rico to determine the output of hookworm eggs from several population groups.

4. Since the knowledge of the output of hookworm eggs of any given population group gives a measure of the potential dissemination of the parasite, this information, as obtained by egg counts, is of importance in planning control measures.

5. In addition, the egg counts of the groups studied gave information relating to the degree of infestation which agreed closely with all other data available, and, we believe, was a true picture of the mass infestation.

6. The data showed that the males had a considerably greater average egg output from ages 20-40 than the females, although in the most heavily infested area the females ~~were~~ output of the was higher.

7. When the data from the egg counts were arranged according to age groups, it was found that the ~~females were~~ output on the youngest groups was low but gradually increased year by year up to ~~nine~~ eight years. From ~~nine~~ eight to seven there was a very rapid increase. During adult life there was not such appreciable

difference in the age groups, but after 50 years there was a distinct increase.

8. The character of the egg output in the different age groups and in the males and females can be correlated with the habits of the people in relation to the visiting of important centers of soil pollution and soil infestation near their houses.

9. The differences in the egg output of the five areas studied agreed with what was known of the conditions in these areas and was definitely correlated with the amount of previous treatment and sanitation.

10. In area C, the most heavily infested of the five areas, egg counts were made before and after treatment, and showed a reduction of 91.8% in the egg output after treatment.

11. The attempt to express the egg output in terms of the number of worms present, by using a factor worked out by Stoll on test cases in Porto Rico, gave figures in essential agreement with all available information.

12. The suggestion is made that the egg counting method be made a regular part of the routine procedure in surveys made before the introduction of control measures or for the evaluation of such measures.

Table IV.

Egg output in Arau before and after treatment

| Age group | Before treatment | | After treatment | |
|---|---|---|---|---|
| | Number of persons examined | Mean egg output per gram of feces | Number of persons examined | Mean egg output per gram of feces |
| 0-9 | 26 | 2450 | 22 | 170 |
| 10-19 | 23 | 8500 | 17 | 1200 |
| 20-29 | 12 | 10700 | 10 | 580 |
| 30-39 | 12 | 8830 | 9 | 260 |
| 40-49 | 6 | 5550 | 5 | 90 |
| 50+ | 7 | 19840 | 6 | 2100 |
| Totals | 86 | | 69 | |

## Literature Cited.


Ashford, B. K. and Gutierrez Iguaravidez, P.

1911. Uncinariasis in Porto Rico. Senate Document No. 808, 61 Congress, 3d Session, Washington. pp. 1-336.

Caius, J. F. and Bhaskar, K. S.

1920-1922. The correlation between the Chemical Composition of Anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. Ind. Jour. Med. Res., vols. VII and VIII.

Darling, S. T.

1920. Observations on the Geographical and Ethnological Distributions of Hookworms. Parasitology, 12, 217-238.

Darling, S. T.

1922. The Hookworm Index and Some Treatment. Amer. Jour. Trop. Med., 2, 327-447.

Darling, S. T. and Smillie, W. G.

1921. Studies on Hookworm Infection in Brazil (First Paper). Monographs of the Rockefeller Institute for Medical Research. No. 14, pp. 1-42.

Darling, S. T., Barber, M. A., and Mackie, M. T.

1920. Hookworm and malaria research in Malaya, Java and the Fiji Islands. Report of Uncinariasis Commission to the Orient, 1916-1917. Publication No. 8, International Health Board, Rockefeller Foundation, New York, pp. 1-191.

Smillie, W. G.

1922 a. Studies on Hookworm Infection in Brazil 1918-1920. Second paper. Monographs of the Rockefeller Institute for Medical Research. No. 17, pp. 1-73.

Smillie, W. G.

1922 b. The results of hookworm disease prophylaxis in Brazil. Amer. Jour. Hyg., 2, 77-96.

Stoll, N. R.

1923 a. Investigations on the Control of Hookworm Disease. XV. An Effective Method of Counting Hookworm Eggs in Feces. Amer. Jour. Hyg., 3, 59-70.

Stoll, N. R.

1923 b. Investigations on the Control of Hookworm Disease. XVI. Amer. Jour. Hyg. 3.